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A new record of the blackfin goby (*Egglestonichthys melanoptera*, Gobiidae) from China, with complete mitochondrial genomes of two *Egglestonichthys* species and their phylogenetic implications

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Abstract

This study documents the northernmost record of *Egglestonichthys melanoptera* in the South China Sea (Hainan Island, China), thereby extending its distribution in the Indo-Pacific region from the Indian Ocean, and confirms the sympatric occurrence of *E. bombylios*. The morphological characteristics of *E. melanoptera* are described in detail, and measurable data is presented. The complete mitochondrial genomes of both species have been presented for the first time (17,743 bp for *E. bombylios*, 17,350 bp for *E. melanoptera*), revealing a conserved gobiid genomic architecture with a pronounced A+T bias (60.3–62.7%) and codon position-specific nucleotide preferences. A further objective of this study was to construct a phylogeny of 110 species of the family Gobiidae, extending previous studies. Phylogenetic analyses resolved *E. melanoptera* within Gobiidae, supported by osteological synapomorphies (single epural; pterygo-phore formula 3-22110), and placed *Egglestonichthys* within the Priolepis lineage. The validity of Benthophilinae remains contentious due to conflicting swim bladder and spine morphology, necessitating integrative reassessment. Comparative mitogenomic analyses reveal that the mitochondrial genome currently attributed to *Oxyurichthys formosanus* in GenBank (accession: KC237282) does not represent the genus *Oxyurichthys* but should be reassigned to *Cryptocentrus yatsui*. A revised diagnostic key has been synthesised, synthesising historical descriptions with novel morphological-molecular data, giving taxonomy for *Egglestonichthys* species. These findings contribute to our understanding of the evolutionary trajectories of gobiid fish, emphasising the synergy of mitogenomic and morphological approaches in resolving complex marine fish systematics.

Key Words

Egglestonichthys melanoptera, Gobiidae, Hainan Island, mitochondrial genome, phylogenetic analysis, South China Sea, taxonomic key

* These authors contributed equally to this work.

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Introduction

The genus *Egglestonichthys* was established by Miller and Wongrat (1979) with *E. patriciae* (type species) from Hong Kong waters. *E. bombylios*, described by Larson and Hoese (1997) as the second congener, has been subsequently reported from the Arabian Sea (Manilo and Bogorodsky 2003), Vietnam (Prokofiev 2016), and Panay Island, Philippines (Motomura et al. 2017). Ni et al. (2024) recently redescribed this species with expanded morphological data from Chinese specimens collected in Dongshan Bay and Niushan Island. Here, we document *E. bombylios* from Hainan Island (Fig. 1A) and report *E. melanoptera* as the third *Egglestonichthys* species in Chinese waters, representing its first national record.

Egglestonichthys melanoptera was originally described as *Callogobius melanoptera* from India's Godavari estuary (Visweswara Rao 1971) and later transferred to *Egglestonichthys* by Larson and Hoese (1997). Its distribution spans eastern India (Godavari estuary; Visweswara Rao 1971), southeastern India (Parangipettai, Pazhayar; Ragul et al. 2024), Vietnam (Nha Trang, Van Phong bays; Prokofiev 2016), the Philippines (Samar Sea), and Papua New Guinea (Orokolo Bay; Larson and Hoese 1997). This study provides the first morphological description of *E. melanoptera* from China based on a Hainan Island specimen, extending its northernmost confirmed range to the southern Chinese coast. Comprehensive morphometrics, meristics, and diagnostic colouration patterns are presented.

The genus *Egglestonichthys* comprises six marine-dwelling species (Ni et al. 2024; Ragul et al. 2024; Fricke and Fong 2025). Morphologically allied with *Priolepis*, *Trimma*, *Trimmatom*, and *Paratrimma* through shared synapomorphies—absence of cephalic lateral-line canals/pores and distinctive cephalo-trunk striping (Winterbottom and Burridge 1992)—its phylogenetic position within Gobiidae has been further resolved through molecular analyses. Thacker and Roje (2011) reconstructed 13 gobiid lineages using mitochondrial (*ND1*, *ND2*, *CO1*) and nuclear (RAG2, Rhodopsin, RNF213) markers across 100 species, while Thacker (2015) later expanded this framework to 19 lineages through integrated chronophylogenetic, biogeographic, and paleontological data. Recent investigations of terrestrial adaptation mechanisms in mudskippers employed five nuclear loci (Gpr85, RAG1, Rhodopsin, Zic1) and mitochondrial cytb/ND1 across 29 oxudercine taxa (Steppan et al. 2022).

Mitogenomic analyses in gobiid phylogenetics remain limited in scope and resolution. Adrian-Kalchhauser et al. (2017) leveraged 51 gobiid mitogenomes to reconstruct phylogenies reconciling phylogenetic patterns with eco-morphological traits. Shang et al. (2022) applied Bayesian inference to 21 mitogenomes, elucidating structural conservation and selective constraints. Most recently, Ni et al. (2024) employed whole-genome sequencing across 20 taxa to resolve the phylogenetic position of *Egglestonichthys* relative to *Glossogobius*, *Priolepis*, *Callogobius*, *Valenciennea*, and *Asterropteryx* clades.

Mitogenomic characterisation of *Egglestonichthys* remains critically understudied, with *E. bombylios* and *E. melanoptera* mitogenomes previously undocumented. Here, we present the first complete mitogenome assemblies for both species, integrating methodological frameworks from Huang et al. (2016), Hanahara et al. (2019), and Shang et al. (2022) while expanding Adrian-Kalchhauser et al.'s (2017) phylogenetic scope. Our analysis positions both *Egglestonichthys* taxa within a mitogenomic phylogeny encompassing 110 gobiids, including

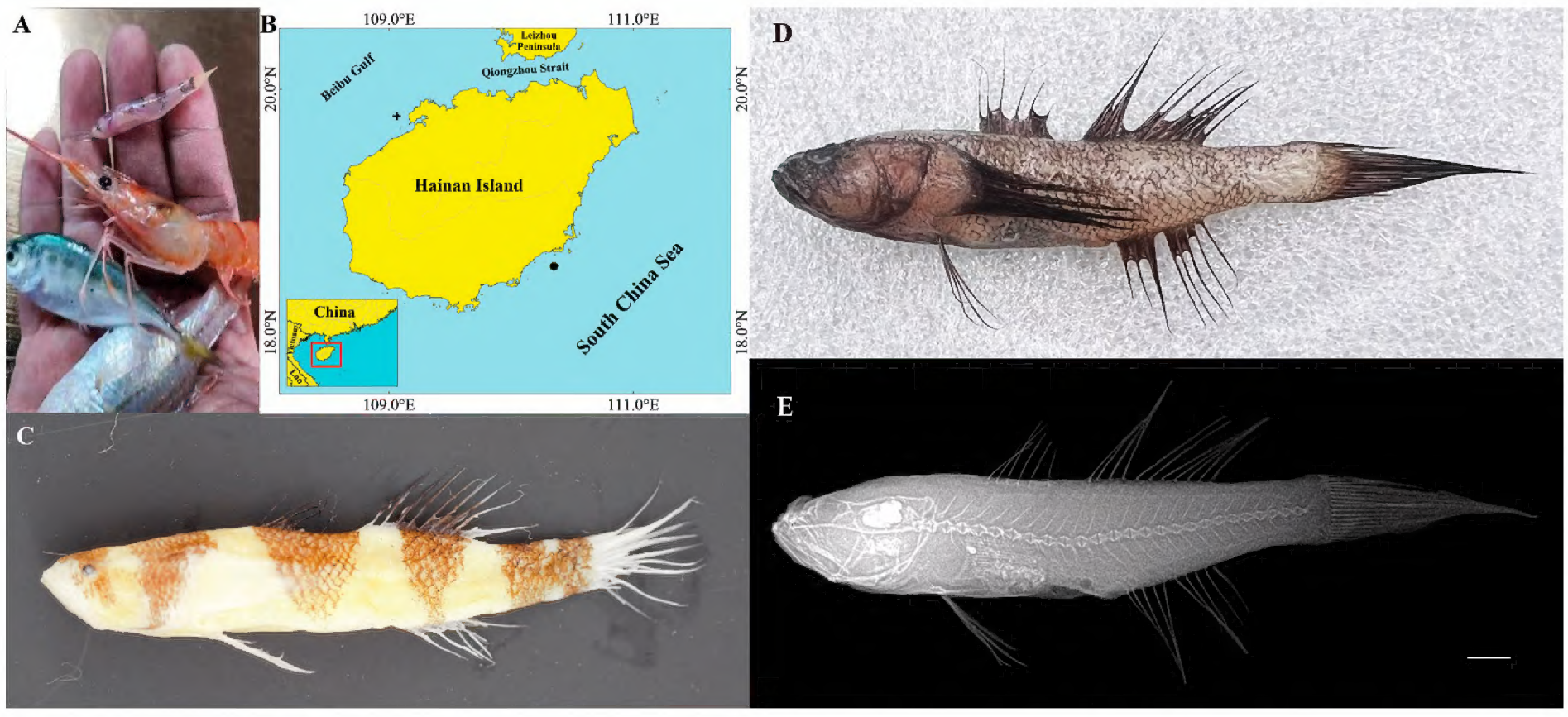


Figure 1. A. Part of the catch, including *E. bombylios*, after a 30-minute haul with a 21-metre bottom trawl off the coast of Hainan Island, China. Photo by Yuange Chen; **B.** Locality map for known records of *E. bombylios* (black crosses) and *E. melanoptera* (solid black circle); **C.** *E. bombylios* Larson & Hoese, 1997 (accession numbers: OR260439, voucher no. E201313, 52mm SL, Dongshan Bay, Fujian Province, China, 12 Sep. 2013). Fresh colouration (**D**) and radiograph (**E**) of *E. melanoptera*, voucher no. ECSFRI 23184, 64 mm SL, Hainan Island, China. Scale bar: 5 mm.

representatives from *Sicydiinae* (4 spp.), *Amblyopinae* (2), *Oxudercinae* (7), *Gobiinae* (44), and *Gobionellinae* (43), with *Siganus guttatus* and *Lactoria diphana* as outgroups. Through gene arrangement profiling and whole-mitogenome phylogenetic reconstruction, we establish evolutionary linkages between molecular phylogenies and ecological trait evolution across the Gobiidae radiation.

Materials and methods

Sample collection and DNA extraction

An individual sample of *E. bombylios* (Fig. 1A) was recorded, and *E. melanoptera* was collected along the coast of Hainan Island, China (118°52'46.087"E, 49°14'50.913"N and 138°50'23.433"E, 30°10'47.634"N, respectively, Fig. 1B) on 7 Dec. 2023. Prior to DNA extraction, specimens were surface-sterilised with absolute ethanol. A 5×5 mm^2 muscle tissue sample excised subjacent to the right dorsal fin from ethanol-preserved specimens was processed using the TIANamp Genomic DNA Kit (TIANGEN, China).

The mitochondrial data of the *E. bombylios* specimen examined in this study (Fig. 1C) was obtained from a previous study (Ni et al. 2024), and together with the *E. melanoptera* specimen in this study (Fig. 1D, E), voucher no. ECSFRI 23184, they were both preserved in 70% ethanol at the East China Sea Fisheries Research Institute, Institute of Fisheries, Chinese Academy of Sciences.

Morphological study

The morphometric protocols followed Wu and Zhong (2008) and Larson (2013). Vertebral counts (hypural included) were derived from radiographic analysis. Supraneural bone arrangement, neural spine positioning, and dorsal pterygiophore configuration were assessed using Akihito et al.'s (1984) notation system, where the first digit-dash combination indicates the interneural space receiving the first spinous dorsal fin pterygiophore. In gobioids, the first interneural space corresponds to the area between neural spines 1 and 2. Predorsal bones (supraneurals) were consistently absent across specimens, aligning with gobioid morphological characteristics (Birdsong et al. 1988). All measurements were obtained from 70% ethanol-preserved specimens, with morphometric values standardised as percentages of standard length (SL) and head length (HL). Specimen preparation included temporary Cyanine Blue 5R staining to accentuate microstructures (Akihito et al. 1993; Saruwatari et al. 1997).

Abbreviations

D—dorsal fin; **A**—anal fin; **P**—pectoral fin; **V**—ventral fin; **C**—caudal fin; **SL**—standard length; **TL**—total length; **HL**—Head length; **TRB**—transverse scale count backward from anal fin origin; **TRF**—transverse scale count forward from anal fin origin; **PD$_1$L**—Pre-first dorsal length; **PD2L**—Pre-second dorsal length; **PPL**—Pre-pectoral fin length; **PVL**—Pre-ventral fin length; **PAL**—Pre-anal fin fin length; **HD**—Head depth; **HW**—Head width; **CPL**—Caudal peduncle length; **CPD**—Caudal peduncle depth; **CPW**—Caudal peduncle width; **D1BL**—First dorsal-fin base length; **D2BL**—Second dorsal fin base length; **ABL**—Anal fin base length; **PL**—Pectoral length; **VL**—Ventral length; **CL**—Caudal length; **IL**—Interorbital length; **SnL**—Snout length; **UJW**—Upper Jaw length; **LJW**—Lower Jaw length; **BD**—Body depth; **BW**—Body width; ***a***—suborbital row; ***pe***—preorbital row; ***ue***—upper orbital row; ***b***—central cheek row; ***d***—lower cheek longitudinal row; ***e***—preoperculomandibular series row; ***ul***—upper lip row; ***f***—medial row.

Mitogenome sequencing and assembly

The mitochondrial genomes were sequenced and assembled following established protocols (Chen et al. 2024; Xu et al. 2024; Yang et al. 2024). DNA libraries were prepared with the Illumina TruSeqTM DNA Sample Preparation Kit (Illumina, USA) following manufacturer guidelines. Novogene Bioinformatics Technology Co., Ltd. (China) conducted sequencing on a DNBSEQ-T7 platform, generating 150 bp paired-end reads yielding ~5 Gb raw data per sample. Data preprocessing involved Fastp v0.23.2 (Chen et al. 2018) with default parameters and quality assessment using FastQC v0.12.1 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Mitogenome assembly employed the FastMitoAssembler pipeline, augmented by GetOrganelle v1.7.6.1 (Jin et al. 2020) and NovoPlasty v4.3.1 (Dierckxsens et al. 2017). Failed assemblies were rescued using Priolepis lineage species mitogenomes (Winterbottom and Burridge 1992) as reference seeds.

Mitogenome annotation and sequence analyses

The complete mitochondrial genome was annotated using MITOS2 (Donath et al. 2019) and Mitoz v3.6 (Meng et al. 2019), with manual validation in Geneious v2021.0.3. PhyloSuite v1.2.3 (Zhang et al. 2020) was employed to quantify base composition, codon usage, and relative synonymous codon usage (RSCU) of protein-coding genes (PCGs). Nucleotide skewness (A+T skew = [A%−T%]/[A%+T%]; G+C skew = [G%−C%]/[G%+C%]) was calculated following Perna and Kocher (1995). Selective pressure analysis through Ka/Ks ratios was conducted for Priolepis lineage species mitogenomes using DnaSP 6.0 (Rozas et al. 2017). Transfer RNA secondary structures predicted by tRNAscan-SE 2.0 (Lowe and Chan 2016; Chan et al. 2021) and MITOS (Bernt et al. 2013) under vertebrate mitochondrial code were visualised through ViennaRNA Web Services (Kerpedjiev et al. 2015; http://rna.tbi.univie.ac.at/forna/).

Phylogenetic analysis

We reconstructed the Gobiidae phylogeny using mitogenomes from 110 species, with *Siganus guttatus* and *Lactoria diaphana* designated as outgroups. Mitogenome sequences retrieved from GenBank (Suppl. material 1: table S1) were processed in PhyloSuite 1.2.3 (Zhang et al. 2020). Thirteen protein-coding genes (PCGs) were aligned codon-wise using MAFFT v7.313 (Katoh and Standley 2013), followed by ambiguous region trimming in Gblocks v0.91 (Castresana 2000). ModelFinder v.2.2.0 (Kalyaanamoorthy et al. 2017) was used to select the best substitution models under the Bayesian Information Criterion (BIC) for maximum likelihood (ML) analysis and the corrected Akaike Information Criterion (AICc) for Bayesian inference (BI) analysis (Suppl. material 1: table S2).

Maximum likelihood analysis employed IQ-TREE v2.2.2 with edge-linked partitioning (5,000 ultrafast bootstrap replicates), while Bayesian inference utilised MrBayes v3.2.7a (Ronquist et al. 2012; two parallel runs; 2×10^6 generations). Final topologies were visualised using iTOL v6 (Letunic and Bork 2016).

Data availability

All data supporting the findings of this study are available in the article and its Suppl. material 1.

Results

Species description

***Egglestonichthys melanoptera* (Visweswara Rao, 1971)**

Diagnosis. D VI-I,10; A I,8-9; P (R/L) 22/21; V I-5; C 17; in 9/8 pattern; branched caudal rays 8/7; longitudinal scales 44; TRB 19; predorsal scales 37; circumpeduncular scales 16. First dorsal-fin pterygiophore formula 3-22110 (Fig. 1D). Vertebrae (including urostyle) 10+16 = 26. Two anal pterygiophores before haemal spine of first caudal vertebra. One epural. Fins black. Caudal fin almost 1/2 SL. Pelvic frenum absent. Body without darker bands. Head sensory papillae developed.

Description. Based on 1 specimen, 84.13 mm SL. Counts and proportional measurements are given in Table 1.

Body moderately robust, rounded anteriorly, compressed posteriorly; dorsal/ventral margins shallowly arched. Depth at first dorsal origin 37.24% SL; caudal peduncle depth 8.73% SL. Head broad (29.62% SL), wider than body depth; snout blunt (1.23 times eye diameter), terminating at eye superior margin. Preopercular width 71.59% HL. Mouth terminal (~45° to body axis), jaws subequal (lower slightly prominent), reaching mid-eye level; upper jaw 41.08% HL. Lips smooth (upper lip 2.2 times eye diameter). Eyes dorsolateral (18.45% HL), no supraorbital ridge. Interorbital convex (25.77% HL; 1.40 times eye diameter; 1.13× SnL) (Table 2). Chin smooth, lacking mental folds/barbels. Paired nostrils separated; anterior tubular, positioned at upper lip posterior margin (short tube, no ventral/anterior orientation); posterior rounded pore-like, adjacent to eye anterodorsal margin (Fig. 2A). Gill openings wide, vertically extended anteriorly to mid-eye.

Body and head fully scaled (cycloid); predorsal region (snout tip to first dorsal-fin origin), operculum, preoperculum, cheek, isthmus, breast, abdomen, and pectoral fin base scaled.

Lateral line absent; cephalic sensory canals and pores absent. Sensory papillae system comprising: suborbital row *a* with four long and four shorter transverse multi-papillate rows; longitudinal row *b* extending medially from first long row of *a* to row *c*; transverse row *c* positioned between longitudinal rows *b* and *d*; longitudinal row *d* running from *c* to upper lip posterior margin (connecting *ul*). Preoperculo-mandibular series *e* uniserial, ascending to *ul* at mouth angle. Preorbital *pe* with dual rows: lower connecting to *ul*, upper reaching anterior nostril base. Thirty-five short *i* rows below *e*; three *i* rows per side extending to mid-isthmus. Mental row *f* longitudinal on anteroventral mandible (not reaching mid-isthmus). No dorsal cephalic papillae observed (Fig. 2C).

Dorsal fins separate, elevated. First dorsal fin short-based, origin posterior to pectoral fin base; soft spines with third spine longest, extending to second dorsal origin. Second dorsal fin longer-based, taller than first, fourth ray longest not reaching caudal base. Anal fin origin opposite

Table 1. Meristic data for *E. melanoptera* from the south-eastern coast of India and China.

Character	Holotype ZSI 7919/2	Type specimens from Larson and Hoese 1997	Specimens from Ragul et al. 2024 (CASMBAURM/2312811-15)	This study (ECSFRI 23184)
SL (mm)	65	68–88	53.9–80.8	84.13
D	VI, I-10	VI, I-10	VI, I-10	VI, I-10
A	I,9	I,9	I,9	I,9
P (R/L)	22/-	20–22/21–22	19–20/20–22	22/21
Segmented Caudal-fin rays	17	9/8	9/8	9/8
Branched caudal rays	-	8/7	8/7	8/7
TRB	-	12–14	-	19
TRF	-	14–17	-	15
Longitudinal scale count	?35	35–45	35–41	44
Pre-dorsal scales	-	29–37	30–32	37
Circumpeduncular Scales	-	14–18	15–16	16

Table 2. Morphometric date characters of *E. melanoptera* from India (Ragul et al. 2024) and China (this study).

Morphometric (mm)	India	China	Morphometric (mm)	India	China
TL	81–129	84.13	D1BL	12.1–13.2	12.23
SL	53.9–83.9	61.31	D2BL	20.6–21.6	22.07
Measurements in % of SL			ABL	14.3–15.4	16.64
HL	27.0–29.1	29.62	PL	29.0–37.7	34.19
PD_1L	36.9–37.5	37.24	VL	23.4–25.1	25.59
PD_2L	55.2–56.9	56.43	CL	49.8–53.9	39.59
PPL	33.2–35.2	34.63	**Measurements in % of HL**		
PVL	31.6–33.1	32.15	Eye diameter	18.1–19.8	18.45
PAL	63.0–66.1	62.93	Orbital diameter	20.9–24.8	24.72
BD at D origin	16.6–23.6	16.34	IL	23.2–26.7	25.77
BD at A origin	17.0–18.9	17.6	SnL	19.5–24.1	22.8
BW at A origin	8.9–10.1	9.17	UJW	42.9–45.4	41.08
CPL	21.1–25.5	22.05	LJW	34.5–43.2	40.2
CPD	9.9–11.1	8.73	HD	52.1–71.4	55.51
CPW	4.8–6.2	5.35	HW	63.7–71.4	60.35

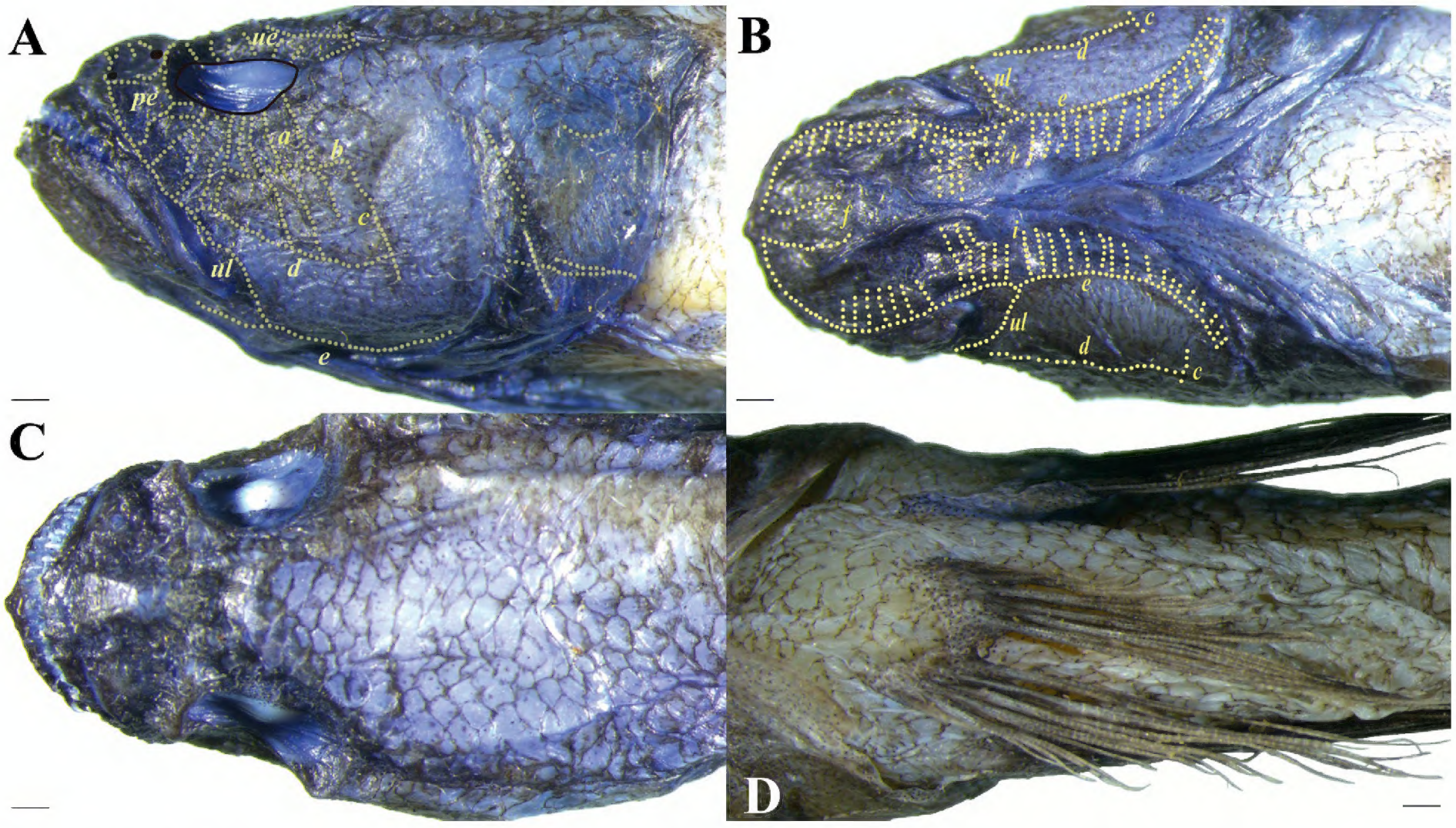


Figure 2. Head of *Egglestonichthys melanoptera* in lateral (**A**), ventral (**B**), dorsal (**C**) view stained Cyanine blue 5R and pelvic fin (**D**), DHS23184, SL 84.13 mm. Scale bar: 1 mm.

second dorsal, base shorter than height; mid-rays not reaching caudal base. Pectoral fins broad and elongated, exceeding head length, posterior margin surpassing anus; no dermal flaps on leading edge. Pelvic fins elliptic-pointed, shorter than pectorals, base 1/5 length; fused into thin-membraned disc. Caudal fin elongate-pointed, longer than head length. Anus positioned anterior to anal fin origin, aligned with third ray of second dorsal fin.

Fresh colouration (Fig. 1D). Body uniformly grey without bands, cephalic region darker; pectoral and caudal fins jet black; dorsal, anal, and ventral fins dark brown to black. Preserved specimens exhibit greyish-brown body colouration with fins maintaining black to rich chestnut-brown tones.

Distribution and ecology. *Egglestonichthys melanoptera* is documented from the Godavari Estuary, eastern India (Visweswara Rao 1971); Parangipettai, southeastern India (50–100 m depth; Ragul et al. 2024); Myanmar waters (50–74 m, muddy substrates; Psomadakis et al. 2020); Vietnam; Samar Sea, Philippines (50–70 m); Orokolo Bay, Papua New Guinea (Larson and Hoese 1997); and the southeastern coast of Hainan Island, China (60 m depth; this study). The species demonstrates an Indo-West Pacific distribution pattern, predominantly inhabiting continental shelf depths of 50–100 m.

Mitochondrial genomic structure and base composition

The complete mitogenomes of *E. bombylios* (17,743 bp; OR260439) and *E. melanoptera* (17,350 bp; PQ273468)

(Fig. 3, Suppl. material 1: table S1) exhibit typical gobiid genomic architecture. All protein-coding genes resided on the heavy strand, excluding *ND6* and eight tRNAs (*trnS*, *trnE*, *trnP*, *trnQ*, *trnA*, *trnN*, *trnC*, *trnY*) on the light strand (Fig. 3; Suppl. material 1: table S3). Four intergenic overlaps (1–7 bp) were detected, with the 7 bp *ND4L-ND4* overlap being the longest. Fifteen intergenic spacers occurred, including species-specific maximum spacers: 835 bp between *COX2* and *trnK* in *E. bombylios* and 1,247 bp between the D-loop and *trnF* in *E. melanoptera*.

Both *E. bombylios* (17,743 bp; A+T=60.3%, A=30.5%, T=29.8%, G=15.0%, C=24.8%; A+T skew=0.111, G+C skew=−0.245) and *E. melanoptera* (17,350 bp; A+T=62.7%, A=31.3%, T=31.4%, G=14.2%, C=23.1%; A+T skew=−0.002, G+C skew=−0.239) exhibit marked A+T bias in mitogenomes, consistent with Gobiinae taxa (Suppl. material 1: tables S1, S4). The third codon positions showed maximal A+T enrichment (69.8% and 74.4%, respectively), aligning with animal mitogenomic trends (Zhang and Hewitt 1997; Satoh et al. 2016). Conversely, the first codon positions displayed minimal A+T content (52.4% and 54.8%), demonstrating codon position-specific nucleotide bias (Suppl. material 1: table S4).

Protein-coding genes and codon usage

The protein-coding genes (PCGs) of *E. bombylios* (11,390 bp) and *E. melanoptera* (11,385 bp) range from 168 bp (*ATP8*) to 1,842–1,851 bp (*ND5*). Ten PCGs in *E. bombylios* and seven in *E. melanoptera* possess complete stop codons, while the remainder exhibit incomplete termini (TA/T in *E. bombylios*; T/A in *E. melanoptera*; Suppl. material 1: table S3), a hallmark of vertebrate mitochondrial transcripts post-polyadenylation (Ojala et al. 1981). Protein-coding genes in *E. bombylios* (A+T skew=−0.066; G+C skew=−0.259) and *E. melanoptera* (A+T skew=−0.094; G+C skew=−0.256) demonstrate pronounced C/A nucleotide bias compared to G/T counterparts (Suppl. material 1: table S4).

Amino acid composition and RSCU patterns in *E. bombylios* (3,795 residues) and *E. melanoptera* (3,793 residues) mitogenomes reveal conserved molecular signatures (Suppl. material 1: table S5, Fig. 4). Both species exhibited leucine as the most abundant amino acid (16.79% and 16.42%, respectively), whereas cysteine showed the lowest frequency: *E. bombylios* at 0.76% and *E. melanoptera* at 0.92%. Shared high-frequency codons include AUU(Ile), CUU(Leu), UUA(Leu), UUU(Phe), and AUA(Met), while species-specific preferences emerged for ACA(Thr) in *E. bombylios* and GGA(Glu) in *E. melanoptera*.

Selection pressure analysis

Selective pressure analysis across 13 mitochondrial protein-coding genes in eight Priolepis lineage species revealed strong purifying selection (Ka/Ks <1 for all genes; Fig. 5), consistent with vertebrate mitogenome evolutionary patterns (Yang and Bielawski 2000). While *nad6* exhibited the highest synonymous substitutions rate (Ka/Ks=0.3029), *COX1* showed extreme constraint (Ka/Ks=0.0339).

Transfer RNA and ribosomal RNA genes

The mitochondrial tRNA complement of *E. bombylios* (66–75 bp; 8.74% total genome) and *E. melanoptera* (65–76 bp; 8.95%) exhibited conserved architectural features (Suppl. material 1: table S3). tRNA clusters showed A+T

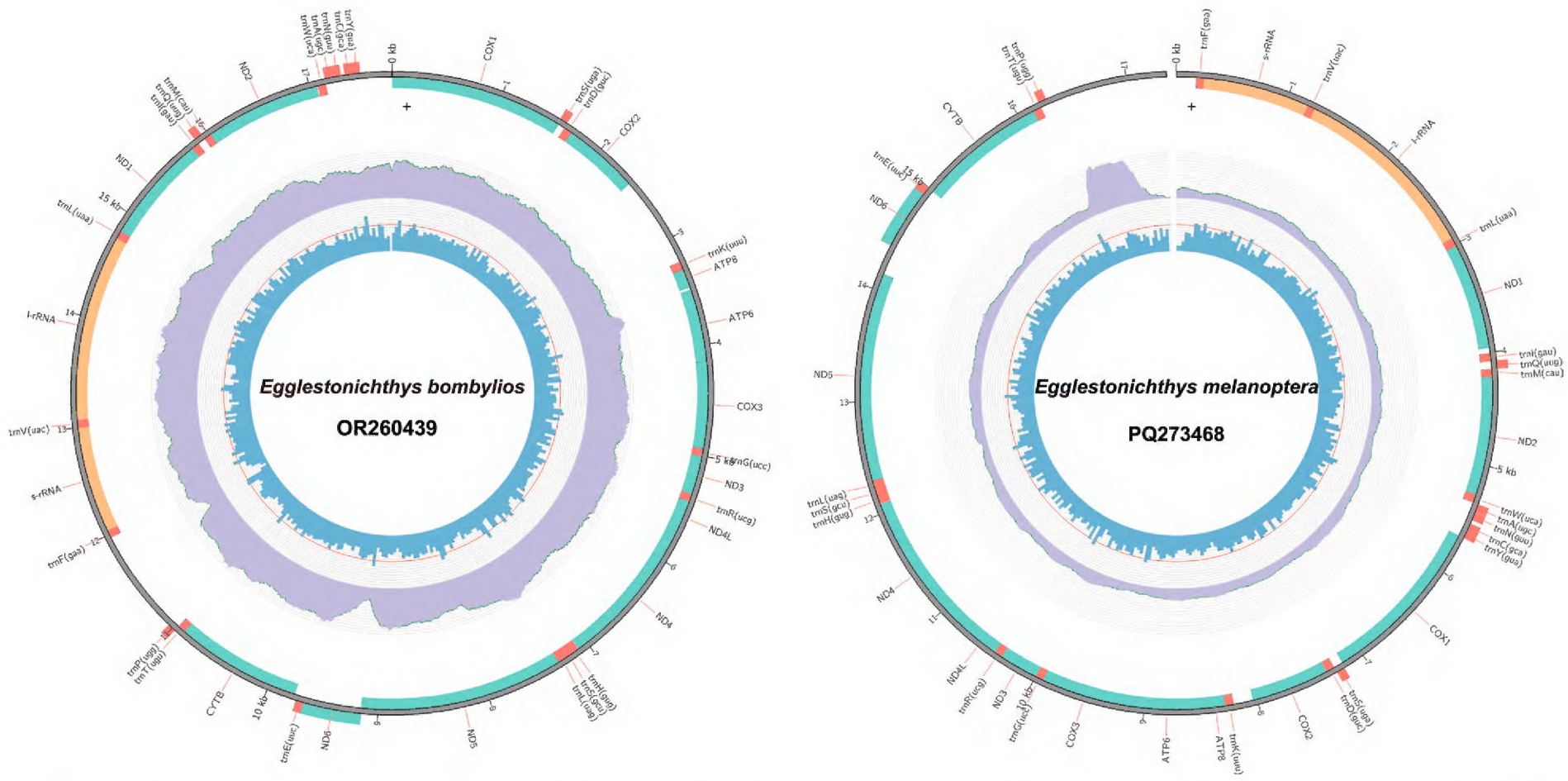
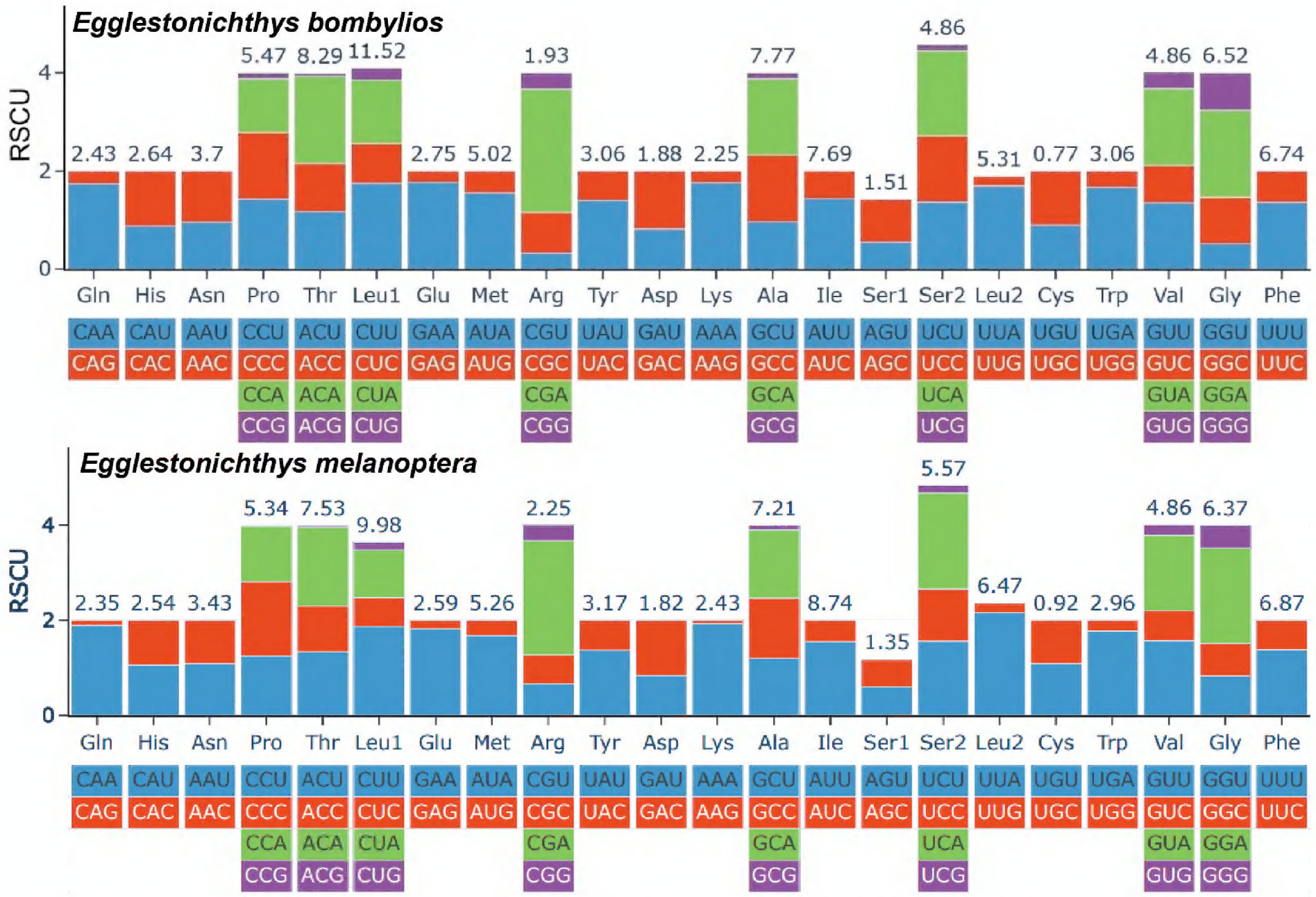


Figure 3. The complete mitogenome of *E. bombylios* and *E. melanoptera*. The middle circles and innermost represent depth distribution and GC content, respectively. The outermost circle shows gene arrangements, with green for PCG fragments, orange for rRNAs and red for tRNAs.


Egglestonichthys bombylios
RSCU
0
2
4
2.43 2.64 3.7 5.47 8.29 11.52 2.75 5.02 1.93 3.06 1.88 2.25 7.77 7.69 1.51 4.86 5.31 0.77 3.06 4.86 6.52 6.74
Gln His Asn Pro Thr Leu1 Glu Met Arg Tyr Asp Lys Ala Ile Ser1 Ser2 Leu2 Cys Trp Val Gly Phe
CAA CAU AAU CCU ACU CUU GAA AUA CGU UAU GAU AAA GCU AUU AGU UCU UUA UGU UGA GUU GGU UUU
CAG CAC AAC CCC ACC CUC GAG AUG CGC UAC GAC AAG GCC AUC AGC UCC UUG UGC UGG GUC GGC UUC
CCA ACA CUA CGA GCA UCA GUA GGA
CCG ACG CUG CGG GCG UCG GUG GGG
Egglestonichthys melanoptera
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0
2
4
2.35 2.54 3.43 5.34 7.53 9.98 2.59 5.26 2.25 3.17 1.82 2.43 7.21 8.74 1.35 5.57 6.47 0.92 2.96 4.86 6.37 6.87
Gln His Asn Pro Thr Leu1 Glu Met Arg Tyr Asp Lys Ala Ile Ser1 Ser2 Leu2 Cys Trp Val Gly Phe
CAA CAU AAU CCU ACU CUU GAA AUA CGU UAU GAU AAA GCU AUU AGU UCU UUA UGU UGA GUU GGU UUU
CAG CAC AAC CCC ACC CUC GAG AUG CGC UAC GAC AAG GCC AUC AGC UCC UUG UGC UGG GUC GGC UUC
CCA ACA CUA CGA GCA UCA GUA GGA
CCG ACG CUG CGG GCG UCG GUG GGG

Figure 4. Relative synonymous codon usage of all PCGs in the mitogenome of *E. bombylios and E. melanoptera*.

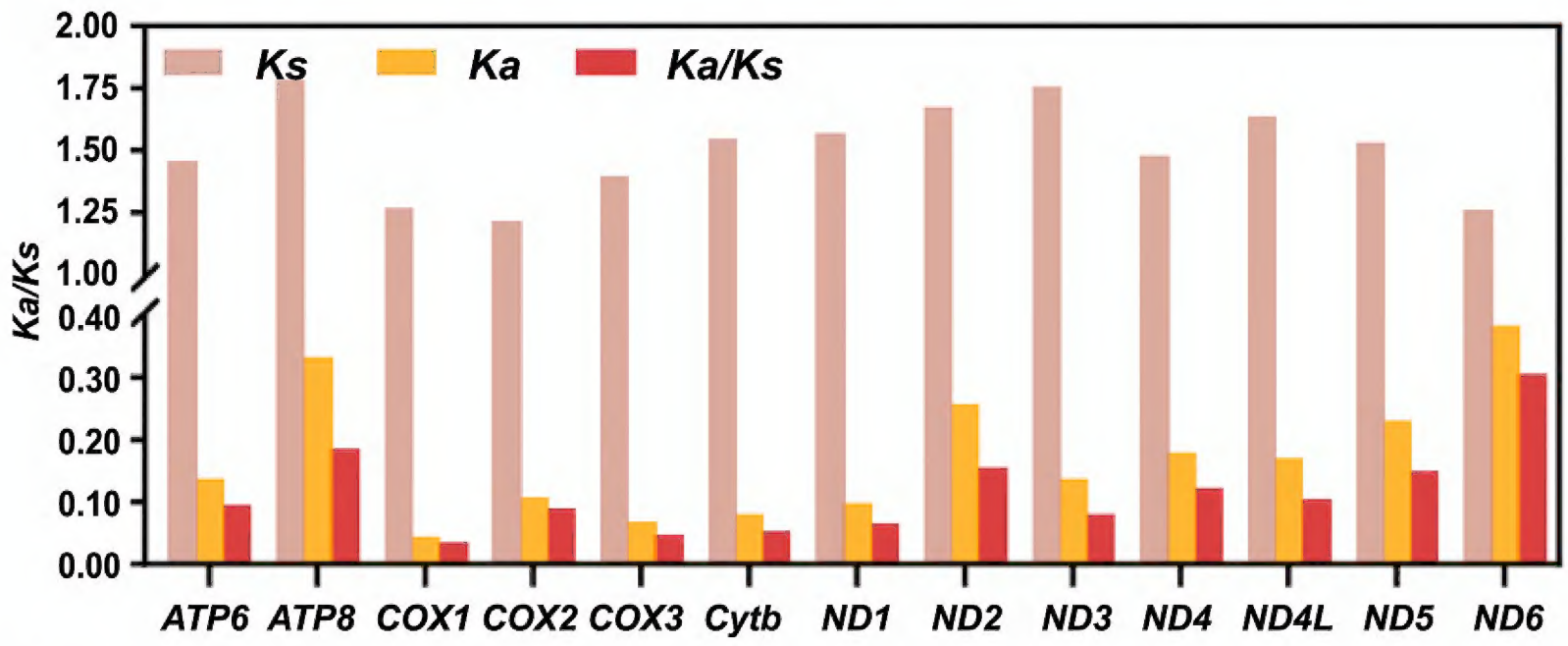

Figure 5. The Ka, Ks, and Ka/Ks values for each PCG from 8 Priolepis lineage species mitogenomes.

biases (57.6% and 63.3%, respectively) with positive skews (A+T=0.007–0.031; G+C=0.035–0.067; Suppl. material 1: table S4). Fourteen tRNAs resided on the heavy (H) strand versus eight on the light (L) strand in both species, mirroring Gobiidae strand distribution patterns (Fu'adil Amin et al. 2020; Harefa and Chen 2024). All tRNAs formed canonical cloverleaf structures except *trnS2*, which lacked the dihydrouridine (DHU) arm (Suppl. material 1: figs S1, S2) — a characteristic feature of teleost mitogenomes (Yang et al. 2018; Wang et al. 2022).

Mitochondrial rRNA genes in *E. bombylios* (12S: 958 bp, 16S: 1,688 bp) and *E. melanoptera* (12S: 946 bp, 16S: 1,682 bp) exhibit conserved genomic arrangement between *trnF*(*gaa*) and *trnL*(*uaa*), separated by *trnV*(*uac*) (Fig. 4, Suppl. material 1: table S3). Both species demonstrate A+T-rich rRNA compositions (*E. bombylios*: 57.8% A+T, skew=0.155; *E. melanoptera*: 59.0% A+T, skew=0.187) with complementary G+C depletion (42.2% and 41.0%, skews=-0.085/-0.081; Suppl. material 1: table S4), revealing pronounced A/C nucleotide preference consistent with vertebrate mitochondrial architectural constraints.

Phylogenetic analysis

Phylogenetic reconstruction (Fig. 6) resolves Gobiiformes into distinct lineages, with Rhyacichthyidae, Odontobutidae, Eleotridae, and Butidae forming independent branch-

es sister to Gobiidae. Within Gobiidae, two reciprocally monophyletic clades emerge, dominated by *Gobionellinae* and *Gobiinae* subfamilies, respectively, demonstrating deep evolutionary divergence within the family.

Within Gobionellinae, the Sicydiinae forms a monophyletic clade sister to Oxudercinae + Amblyopinae. The remaining Gobionellinae resolve into three distinct lineages: 1) Acanthogobius-dominated Northern Pacific Group; 2) Acanthogobius-Mugilogobius Complex (Mugilogobius Group); and 3) Pomatoschistus-associated Sand Gobies clade, encompassing Mediterranean and Northeast Atlantic taxa.

Within the clade of Gobiinae, there are five groups or branches. Firstly, the new record species and tiny banded gobies group together to form the Priolepis lineage. Inshore gobies group together to form the Glossogobius lineage. Burrowing paired gobies, reef shrimp gobies, flapheaded lagoon gobies and silt shrimp gobies form a Gobiopsis-Cryptocentrus branch, but a Gobionellinae of *Schismatogobius ampluvinculus* also clusters with this branch; finally, coral gobies cluster with taxa previously classified within Benthophilinae (Neilson and Stepien 2009; Thacker 2015) and additional Gobionellinae representatives, collectively forming the Gobiodon-Gobius lineage.

The Gobiinae clade resolves into five evolutionarily distinct lineages: 1) the Priolepis lineage comprising newly recorded species and tiny banded gobies; 2) inshore gobies forming the Glossogobius lineage; 3) a Gobiopsis-Valenciennea complex incorporating burrowing paired gobies, reef shrimp gobies, and flapheaded lagoon gobies; 4) a Gobiopsis-dominated assemblage incorporating silt shrimp gobies, lagoon specialists, and the Cryptocentrus group nested within; and 5) the Mugilogobius-Gobiodon transsubfamilial cluster containing coral gobies and heterofamilial elements (Benthophilinae: Mediterranean/Eastern Atlantic gobies; Gobionellidae: Mugilogobius Group).

Discussion

Phylogenetic analyses confirm *E. melanoptera*'s placement within Gobiinae, distinguished from Gobionellinae by diagnostic osteological features: single epural (vs. two in Gobionellidae) and first dorsal pterygiophore formula 3-22110 (vs. 3-12210) – characteristic configurations aligning with core Gobiidae synapomorphies (McKay and Miller 1997; Carpenter and Murdy 2001; Thacker 2009; Pezold 2011). Notably, the central cheek row (*b*) on the specimen's head fails to reach the operculum, with this morphological feature potentially resulting from trawling-induced damage to the sensory papillae during collection.

Phylogenetic analyses resolve *Egglestonichthys* species within the Priolepis lineage (*P. cincta* + *Larsonella pumilus*; Hanahara et al. 2019), forming a distinct clade sister to *Gobiinae*. This lineage diverges from Gobiinae's Glossogobius-Bathygobius complex (Atlantic/Eastern Pacific colonisers via multiple dispersal routes; Thacker 2015). Contrastingly, other Gobiinae lineages – including *Asterropteryx*, *Callogobius*, *Cryptocentrus*, *Gobiopsis*, and *Gobiodon* – exhibit Indo-Pacific endemism, collectively forming the subfamily's most speciose radiation.

Phylogenetic analyses reveal unexpected systematic relationships: Benthophilinae (*Neogobius melanostomus* + *Ponticola kessleri*) and Gobionellidae (*Schismatogobius ampluvinculus*) form a Mugilogobius-Gobius clade sister to Gobiodon lineages, while silt shrimp gobies cluster with lagoon gobies in a Cryptocentrus-Gobiopsis clade. This topology corroborates Thacker's (2015) Indo-Pacific origination hypothesis, demonstrating three Cenozoic dispersal trajectories: 1) Mugilogobius (~48.7 Ma) and Gobius (~45 Ma) lineages diverged from Gobiinae, maintaining Indo-Pacific endemism; 2) Cryptocentrus and Gobiopsis radiated across Indo-Pacific habitats following ~36.2 Ma divergence.

The taxonomic validity of Benthophilinae remains debated, exhibiting diagnostic morphological divergence from Gobiinae through swim bladder absence and elongated fin spines (Miller 1986; Pinchuk 1991; Simonović et al. 1996). Definitive systematic resolution requires expanded molecular datasets integrated with pan-taxonomic morphological comparisons to validate its subfamilial distinctiveness.

Existing studies (Nguyen et al. 2022) have indicated that the mitogenome of *Oxyurichthys formosanus* (GenBank accession number: KC237282) deposited in GenBank does not belong to the genus *Oxyurichthys*, which has led to subsequent phylogenetic misinterpretations of this species (Adrian-Kalchhauser et al. 2017). The *CO1* fragment from KC237282 completely matches the *CO1* sequence of *C. yatsui* (GenBank accession number: KU944915). In conclusion, phylogenetic evidence suggests the KC237282 sequence may represent *Cryptocentrus yatsui*, currently misassigned to *Oxyurichthys formosanus* in the NCBI taxonomy database.

Notably, phylogenetic analyses revealed that *Schismatogobius ampluvinculus* (Gobionellinae) forms a clade with silt shrimp gobies, anomalously nested within the Gobiinae. This phylogenetic incongruence mirrors patterns observed in recent molecular systematic revisions (McCraney et al. 2025).

The sand gobies' lineage (*Pomatoschistus microps*, *P. minutus*, *Gobiusculus flavescens*) is confirmed within Gobionellidae, validating Thacker and Roje's (2011) biogeographic hypothesis. Phylogenetic analyses further resolve: *Lesueurigobius friesii* clustering with *Valenciennea longipinnis* and *Amblygobius phalaena* to form a monophyletic Valenciennea lineage; *Crystallogobius* and *Gobiusculus* nested within the Pomatoschistus radiation; *Exyrias* and *Parachaeturichthys* positioned in the Gobiopsis clade; *Bathygobius* emerging as sister to *Glossogobius*, constituting a distinct Glossogobius evolutionary unit.

Our study reveals broad congruence with McCraney et al. (2020) in the phylogenetic relationships of Gobiaria families and lineages, supporting the current framework of gobioid relationships proposed by Gierl et al. (2022). However, key divergences emerge when compared to earlier studies: Amblyopinae, previously identified as a sister clade to Oxudercinae (Tornabene

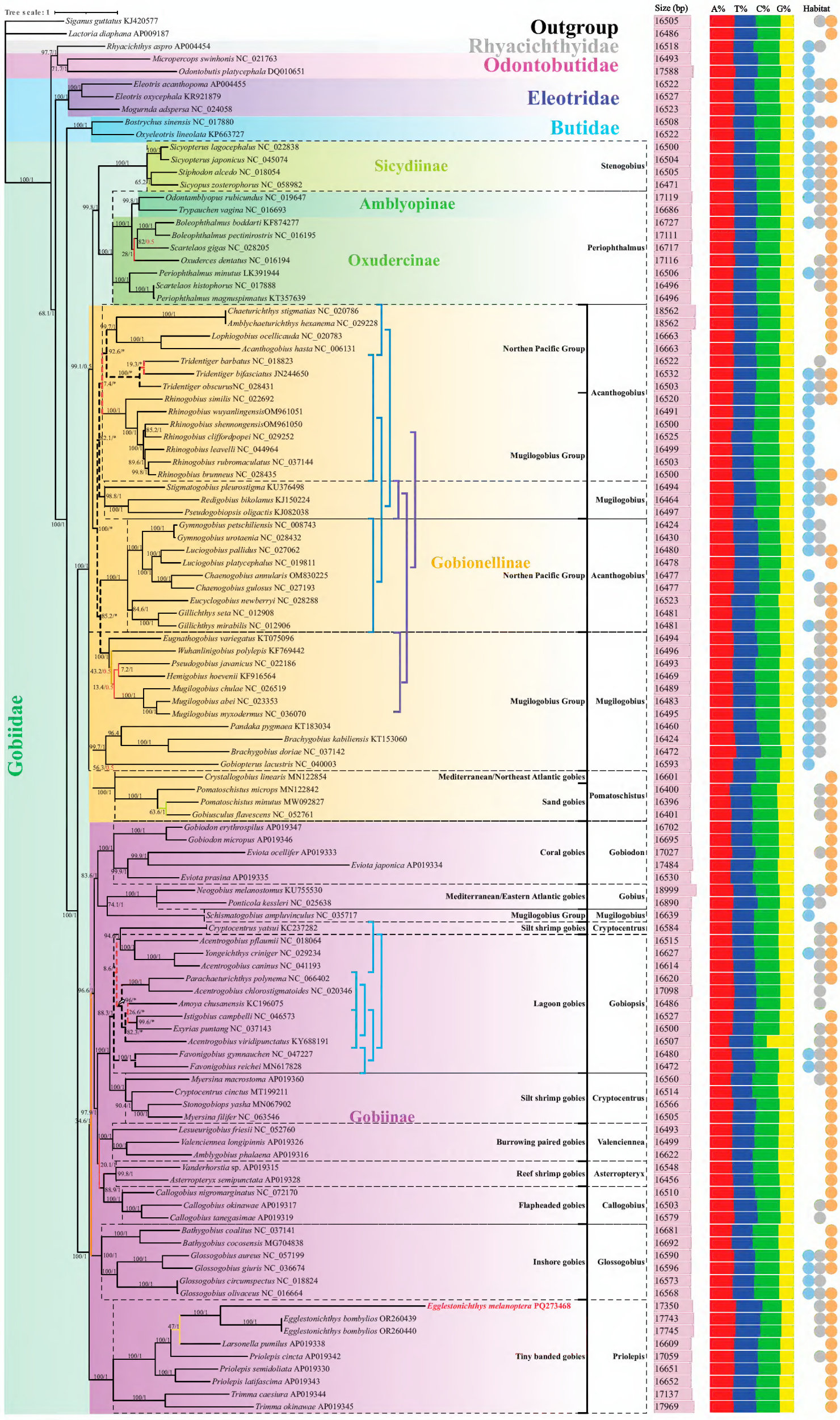

Figure 6. Phylogenetic tree of Gobiidae constructed based on the amino acid sequences of 13 protein-coding genes analysed by Bayesian inference (BI) and maximum likelihood (ML) and their groupings, clades, ecotypes and mitochondrial lengths. Numbers above branches indicate ML bootstrap and Bayesian posterior probabilities, respectively; "*" indicates absent from maximum clade credibility tree. The red, blue, green, and yellow squares represent the proportion of A, T, C, and G content in the sequence, respectively. The blue circle means freshwater, the grey circle means brackish, and the orange circle means marine. Blue and purple square brackets indicate the clustering relationship of sequences in the BL tree.s.

et al. 2013), is resolved here as nested within Oxudercinae, while Eleotridae clusters with Butidae and Gobiidae, contrasting with its traditional grouping alongside Odontobutidae and Rhyacichthyidae (Chakrabarty et al. 2012). Notably, mitochondrial 12S rRNA analyses (Jeon et al. 2021) align with our findings at the family level but exhibit minor topological variations within Gobiidae lineages, underscoring the limitations of single-marker approaches for fine-scale resolution. These discrepancies highlight the impacts of expanded taxon sampling, multilocus data integration, and adaptive morphological convergence on phylogenetic inference.

Distribution and habitat

The newly recorded species inhabits brackish estuaries with strong tidal flushing, mirroring ecological preferences of congeners. Documented from the southeastern and northwestern coastal waters of Hainan Island – both mangrove-fringed estuarine systems (Li et al. 2016; Bai et al. 2021; Wen et al. 2023) – its distribution appears strongly associated with flourishing mangrove ecosystems, suggesting potential ecological dependency on these transitional marine-terrestrial habitats.

The genus *Egglestonichthys* comprises only six known species. *E. patriciae* was initially reported from Hong Kong, China, by Miller and Wongrat (1979) and remained the sole recorded species in Chinese waters until recently. *E. bombylios*, previously documented in Vietnam (Prokofiev 2016), has now been reported from China (Ni et al. 2024; this study), indicating its broad distribution across the Central-West Pacific. Similarly, *E. melanoptera*, first described from India (Visweswara Rao 1971), was later recorded in Vietnam (Larson and Hoese 1997), Myanmar (Psomadakis et al. 2020), and recently along the southeastern Indian coast (Ragul et al. 2024). Its discovery on Hainan Island, China, further confirms an Indo-West Pacific distribution. However, *E. melanoptera* remains poorly understood in China compared to *E. bombylios*. This knowledge gap arises from two factors: 1) their small body size (often overlooked in mixed catches or misidentified as other taxa) and 2) scarcity in trawl surveys due to technical limitations, resulting in fragmentary specimens that are frequently discarded.

Building upon the taxonomic framework established by Larson (2013) and integrating contemporary morphological data (Allen et al. 2020; Fujiwara et al. 2020; Ni et al. 2024; this study), we propose the following diagnostic key for species delimitation within the genus:

Key to the species of *Egglestonichthys*

1 Pelvic frenum absent; body without darker bands; mouth large and reaching to well below eye 2
– Pelvic frenum present, sometimes thin and easily damaged; body with darker bands; mouth small to large 4
2 Body whitish with yellow patches; lateral scales 25 ..... *E. fulmen* (Okinawa Island, Ryukyu Islands, Japan)
– Body plain red or dark brown to black without yellow patches, with at least 27 lateral scales or more 3
3 Body generally plain, dark brown to black; lateral scales 31–45; predorsal scales 29–37; anal rays 8–9 ..... *E. melanoptera* (India to Myanmar; Vietnam; South China Sea)
– Body generally red to reddish brown; lateral scales 27–28; predorsal scales 13–18; anal rays 7 ..... *E. rubidus* (Milne Bay, Papua New Guinea; Yaeyama Islands, Japan)
4 Lower 3–7 pectoral-fin rays free from fin membrane; caudal-fin elongate and pointed, much longer than head; lateral scales 22–25 ..... *E. ulbubunitj* (Northern Territory, Australia)
– All pectoral-fin rays contained within membrane; caudal fin not elongate and pointed; with at least 30 lateral scales 5
5 Papilla rows on head raised on fleshy ridges, especially around jaws, anterior cheek and snout; lateral scales 31–35; eyes small (9–13 in HL) ..... *E. bombylios* (India to Philippines, East China Sea to Australia)
– Papilla rows on head not raised on fleshy ridges; lateral scales 40; eyes moderate (3.5 in HL) ..... *E. patriciae* (South China Sea)

Conclusion

This study reports the northernmost distribution of *Egglestonichthys melanoptera* in the South China Sea (Hainan Island, China), extending its Indo-Pacific range from the Indian Ocean, alongside confirming the sympatric occurrence of *E. bombylios*. Mitochondrial genomes of both *Egglestonichthys* species were sequenced, with phylogenies validating: (1) *E. melanoptera*'s placement in Gobiidae via osteological synapomorphies (single epural; pterygiophore formula 3-22110); (2) *Egglestonichthys* clustering within the Priolepis lineage; and (3) comparative mitogenomic analyses revealing that the mitochondrial genome (GenBank accession: KC237282), currently labelled as *O. formosanus*, should be reclassified to *C. yatsui*. Previous classifications placed these taxa in Benthophilinae based on conflicting swim bladder/spine morphology, but our results reclassify them within Gobiinae, demanding integrative reassessment. A revised diagnostic key for *Egglestonichthys* integrates historical descriptions with novel morphological-molecular data.

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Author contributions

Jia-Jie Chen conducted specimen collection, taxonomic identification, morphometric analysis, photographic documentation, cartographic visualisation, molecular genetic experiments, and manuscript preparation, with specific responsibility for standardised measurement protocols and methodological schematisation. Shen Zeng and De-Yuan Yang performed mitogenome annotation, assembly, and phylogenetic reconstruction. Roland Passmore conducted linguistic editing, participated in scientific discourse refinement, and critically reviewed the manuscript. Wei Tian, Yi-Ming Luo, and Yuan-Ge Chen contributed to field collection and specimen curation. De-Yuan Yang, Jun-Sheng Zhong, and Meng-Ling Yang oversaw research design, funding acquisition, and extramural mentorship. Jin-Qing Ye and Yuan-Ge Chen provided critical project oversight and funding coordination. Jia-Jie Chen and Shen Zeng share co-first authorship. All authors have reviewed and approved the final manuscript for publication.

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Supplementary material 1

Supplementary information

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Data type: zip

Explanation note: **table S1.** Mitochondrial sequences used in the phylogenetic analysis as shown in this study. **table S2.** Best partitioning schemes and models based on different datasets for Bayesian inference (BI) and maximum likelihood (ML) analysis. **table S3.** Information on each gene fragment of *E. bombylios* and *E. melanoptera*. **table S4.** Base composition of the *E. bombylios* and *E. melanoptera* mitochondrial genome. **table S5.** Codon number and RSCU of *E. bombylios* and *E. melanoptera* mitochondrial PCGs. **figure S1.** Secondary structure of the 22 tRNA genes of the mitochondrial genome of *E. bombylios*. **figure S2.** Secondary structure of the 22 tRNA genes of the mitochondrial genome of *E. melanoptera*.

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